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Measurement of Sunflower Seed Respiration Rates and Quotients by Gas–Solid Chromatography in a Closed System[a]

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Sunflower seed respiration rates and respiratory quotients (RQ) were measured by gas–solid chromatography coupled to a closed system which contained the seed sample. By using a gas sampling valve fitted with a 0.5 ml sample loop, head-space sampling is eliminated. The system is reliable, reproducible, and the data can be obtained in short time periods, as O_2 and CO_2 can be measured simultaneously. With this procedure, a 10°C increase in temperature produced about a two-fold increase in respiration rates ($\mu l\, CO_2\, h^{-1}\, g^{-1}$ DM) in seed samples at the same moisture level (13%); while a three-fold increase in seed moisture (7% *vs* 20%) produced a 148-fold increase in respiration rates at 42°C. High RQ values indicated that high temperatures and low seed moisture may have limited O_2 uptake by sunflower seed. Under other conditions, the seed respired aerobically and used carbohydrates as an energy source (RQ=1). Germination test indicated that exposure of sunflower seed to very low O_2 and high CO_2 levels for 24 h did not adversely affect seed viability. This procedure was successfully applied to obtaining respiration data for soya beans and fescue grass seed.

Keywords: Respiration rates; RQ values; sunflower seed; closed system; gas sampling valve; gas chromatography.

1. Introduction

The gas chromatograph has been described as a respirometer, and one advantage is the measurement of O_2 and CO_2 simultaneously.[1] Systems of this type have been used recently to determine O_2 and CO_2 respiration of dry beans, soils, fresh fruits and aerated cultures.[2–5] Most of these systems employed single columns packed with Porapak Q and Porapak N which separate CO_2 and O_2[1,3] and Chromosorb 102 which separates N_2, O_2 and Ar.[5] Other systems used two columns in series packed with silica gel and molecular sieves.[2,4] However, most seed respiration measurements have been done manometrically and required several days to obtain the data.[6–8]

This paper describes a simple method to determine sunflower seed respiration rates and respiratory quotients (RQ) using gas–solid chromatography with a single column packed with Carbosieve S-II and coupled to a closed system which contained the seed sample. Inherent errors usually associated with gas analysis by head-space sampling and subsequent syringe injections are eliminated because sample injections are done with a six-port gas sampling valve fitted with a 0.5 ml sample loop. Once the apparatus has been set up, operation is simple, and results are reliable, reproducible, and can be obtained in relatively short time periods.

[a]Mention of a company or trade names is for descriptive purposes only and does not imply endorsement by the US Department of Agriculture.

2. Experimental

2.1. Plant materials

Sunflower seed (*Helianthus annus* L.) used in this study were high-oil type grown in North Dakota (81–82 crop). Cleaned whole seed were exposed to relative humidities of 63 and 82% at 20°C for 2 weeks in environmental chambers to maintain seed moistures of 7 and 10%. Seed moisture of 13% was obtained by exposure of the seed to 93% r.h./20°C for 24 h. Seed were equilibrated to a moisture of 20% by soaking the seed in distilled water for 1.5 h. Excess water was removed with an air-gun. Seed moistures were determined by AOCS methods (9) and occasionally germination tests were performed after respiration measurements.

2.2. Respiration apparatus

Seed respiration was measured in a closed system (Figure 1). About 130 g of whole, cleaned sunflower seed were put into a 2.7 cm (i.d.)×60 cm glass chromatography (g.c.) column (LKB, Stockholm, Sweden). Both plastic end fittings of the column were adapted to fit 0.32 cm (o.d.) copper tubing which connected the seed-column (A in Figure 1) to a Valco gas sampling valve (6-port, Supelco Inc., Bellefonte, Pensylvania) mounted on the gas chromatograph (C in Figure 1). A small drying tube was made from 0.7 cm (o.d.)×11.5 cm clear plastic tubing with brass nuts and ferrules. The tube was filled with crushed drierite (indicating), plugged with glass wool, and put in line before the gas sampling valve (B in Figure 1). The glass column was wrapped with a heating-tape and aluminium foil to maintain constant seed-mass temperature which was regulated with a rheostat (E in Figure 1). A small pump (Air Cadet, Cole Palmer, Chicago, Illinois) was also connected in the line (D in Figure 1) in order to circulate the closed atmosphere of the seed sample through the sample loop (0.5 ml) fitted on the gas sampling valve. The column was allowed to equilibrate for 1 h to the desired temperature prior to putting in the seed sample. As soon as the seed sample was put into the column, all fittings were quickly tightened, the pump turned on, and the first results (after 5 min) were designated as zero-time for respiration. Gas samples could then be conveniently analysed at various time intervals as the seed respired.

2.3. Gas chromatographic conditions

Gas analysis was performed by gas–solid chromatography using a Hewlett Packard 5750 gas chromatograph. The column, 0.32 cm (o.d.)×213.3 cm premium grade stainless steel, was packed with Carbosieve S-II, 100–120 mesh (Supelco Inc, Bellefonte, Pensylvania). Helium was carrier gas with a flow rate of 16 ml min^{-1}. Thermal conductivity detectors (TC) were operated at 170 Ma. The

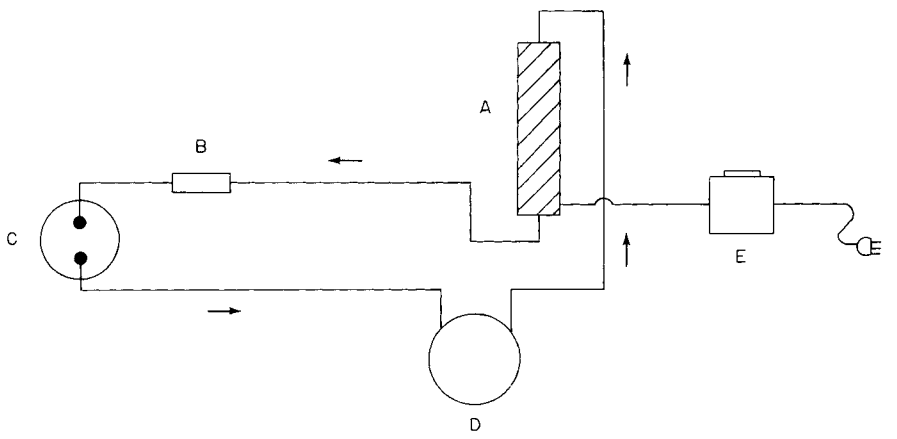

Figure 1. The closed system apparatus. Air flow is indicated with arrows. A, Seed column (27×60 cm) and heating tape; B, drying tube; C, sampling valve on g.c. with 0.5 ml sample loop; D, pump; E, rheostat.

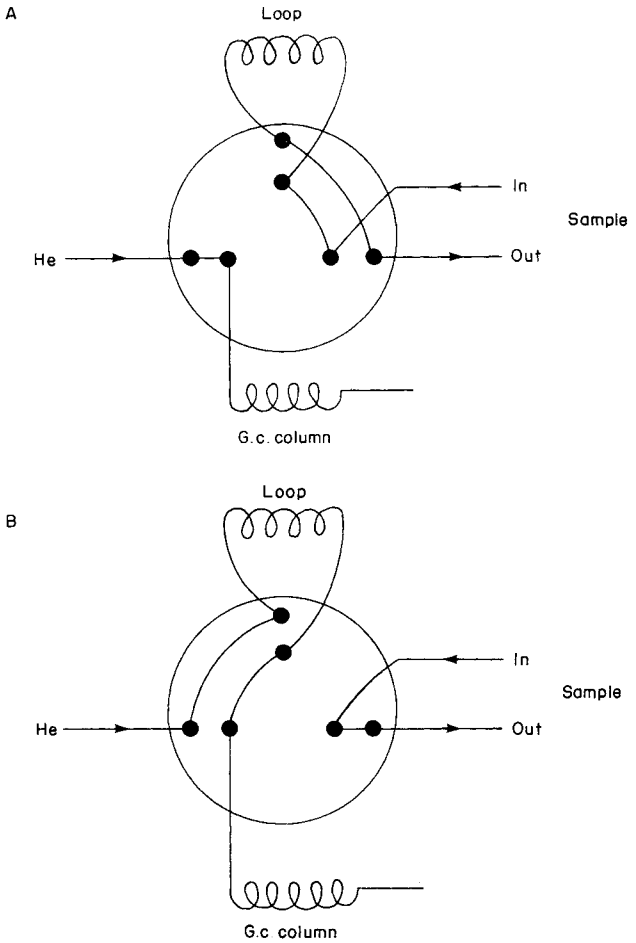

Figure 2. Diagram of sample valve operation (C in Figure 1). A: Gas sample from seed column is flushed through sample loop; B: carrier gas (He) is diverted through sample loop and injects gas on to g.c. column.

gas sampling valve oven was maintained at 95°C. Injector-port temperature was maintained at 100°C and the TC oven at 200°C. The column oven was operated at room temperature (30°C, oven-fan on with no heat, oven door opened) for 7 min then programmed at 40°C min^{-1} to 160°C and held at the upper limit for 7 min. Prior to injection, the pump was turned on for 5 min to flush the sample loop (0.5 ml) with sample gas from the seed column and to ensure uniform gas-mixing. Operation of the gas sampling valve with the 0.5 ml sample loop is shown in Figure 2.

Using TC detection, there is a tendency for sharp fluctuations to occur in the base-line because of heating differential between the two g.c. columns during the temperature-programme mode. This problem can be greatly minimised by operating the column oven at 160°C isothermally for 25 min prior to each sample injection. Once the base-line has stabilised, then the column oven can be cooled to room temperature and normal operation conducted with minimal base-line fluctuation. Because this procedure is necessary, the minimum time between gas measurements is about 50 min.

2.4. Gas analysis and void volume determination

Gases (O_2 and CO_2) were quantitated by normalisation with standard gas mixtures with N_2 as internal standard (Scotty I, Supelco Inc, Bellefonte, Pensylvania). Peak areas were determined and quantitated with a Spectra Physics System I computing integrator. With the 0.5 ml sample injection,

the lower limit of detection for O_2 and CO_2 was 0.4% (vol %) or approximately 2.0 μl under the conditions described.

Volume % CO_2 was converted to total μl CO_2 produced by the seed as a function of time to report respiration rates as μl $CO_2 h^{-1} g^{-1}$ DM (dry wt of seed). Total amounts of O_2 and CO_2 at various times during seed respiration were computed by multiplying gas vol % by the total void volume of the system (167 000 μl). The void volume of the system was determined by adding the computed volumes of all connecting tubes, fittings and head-space volume above the seed sample (130 g) on the column to the void-volume of the seed-mass. The seed-mass void volume was determined by placing 130 g of seed in a large graduated cylinder to obtain the free-fall bed volume. Dry silicic acid 100–200 mesh (Bio-Sil A, Bio-Rad Laboratories, Richmond, California) was added to the seedbed. Gentle shaking with a vortex mixer caused the silicic acid to completely fill the void spaces between the seeds. The seed and silicic acid were separated by shaking over a wire screen and the recovered silicic acid volume measured. This measurement represented a good estimate of the seed-mass void volume. Sunflower seed may swell as they take up moisture and perhaps affect bed- and void-volumes; however, this was found not to be the case. Measurement of bed- and void-volumes at several seed moisture levels (6, 9, 11, 13 and 20%) yielded average seed bed-volumes of 301.4±4.4 ml and void-volumes of 117.8±3.5 ml. Therefore, based on these data, total void volume was calculated to be 167 000 μl and used for all respiration measurements.

Respiratory quotients (RQ) for sunflower seed were computed from the ratio of vol % CO_2 produced to vol % O_2 consumed during maximum CO_2 evolution and O_2 uptake or at any time interval during seed respiration.

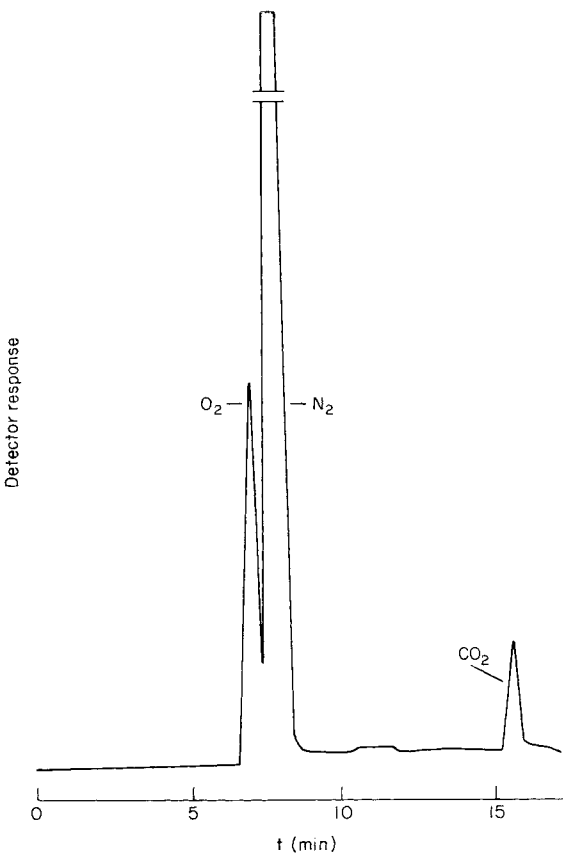

Figure 3. Gas chromatographic profile showing the resolving capability of Carbosieve S-II for O_2, N_2 and CO_2 in sunflower seed respiring at 42°C and 13% moisture for 2 h.

3. Results

3.1. Gas resolution, O_2 uptake and CO_2 evolution

A typical chromatogram of the resolution of O_2 and CO_2 on Carbosieve S-II is shown in Figure 3. Since good resolution of these gases can always be acheived with Carbosieve S-II under the conditions described, accurate quantitation of O_2 and CO_2 is possible. Both gases are quantitated simultaneously which offers a distinct advantage over manometric techniques used to measure seed respiration. Oxygen uptake and CO_2 evolution as a function of time (h) are shown for sunflower seed (13% moisture) respiring at 32 and 51°C (Figures 4 and 5). Average RQ values at 32°C were

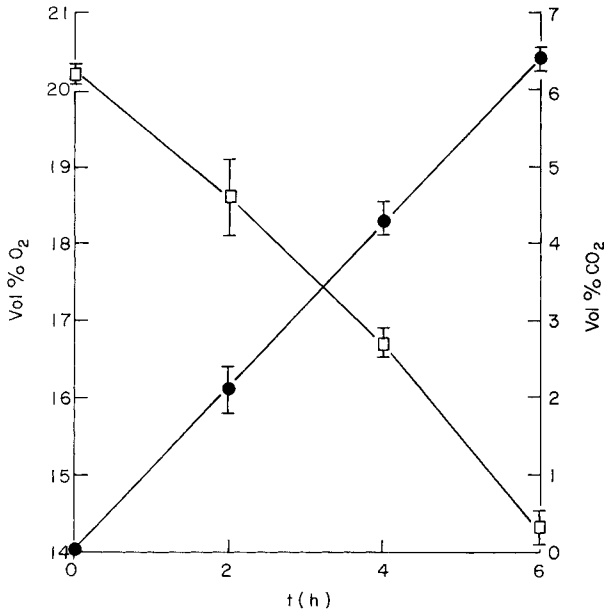

Figure 4. Oxygen uptake and CO_2 evolution in sunflower seed respiring at 32°C. Average values of 4 determinations and ranges are shown.

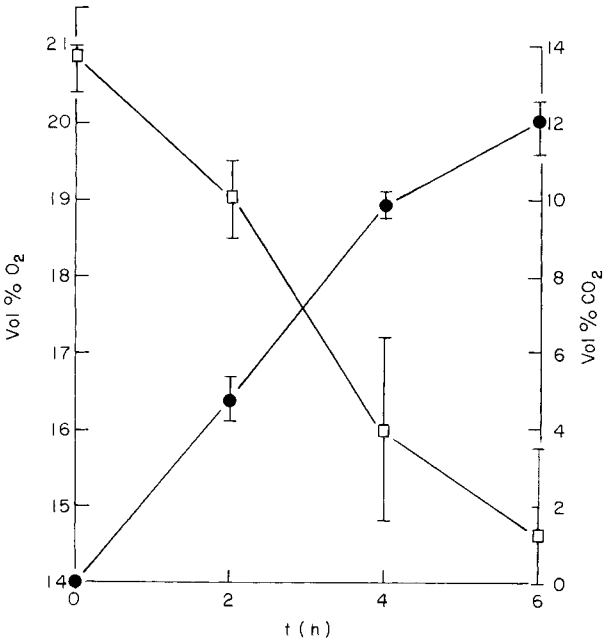

Figure 5. Oxygen uptake and CO_2 evolution in sunflower seed respiring at 51.5°C. Average values of 4 determinations and ranges are shown.

computed to be near unity during any time span measured, while at 51°C, RQ values were much higher than unity.

3.2. Temperature effects on respiration

The effects of seed-mass temperature on respiration rates of sunflower seed equilibrated to 13% moisture is shown in Table 1. A 10°C rise in seed-mass temperature (32 to 42°C) resulted in a two-fold increase in respiration rates of these seed (Table 1). However, the rates only increased 37% from a seed-mass temperature of 42 to 51°C. Respiratory quotients were about unity in seed respiring at 32 and 42°C, but almost doubled at 51°C (Table 1).

Table 1. The effect of temperature on sunflower seed respiration rates and RQ values

°C	Rate[a]	RQ[b]
51.5	37.7±1.1	1.9±0.6
42.0	27.5±2.1	1.3±0.2
32.0	15.8±0.2	1.1±0.2

[a] μl CO_2 h^{-1} g^{-1} DM. Seed moisture was 13% at all temperatures measured.

[b] Ratio of vol % CO_2/vol % O_2. Values are averages and standard deviations for four determinations.

Table 2. The effect of sunflower seed moisture on respiration rates and RQ values

% moisture	Rate[a]	RQ[b]
7	0.41±0.2	2.4±0.8
10	4.1±0.1	1.2±0.1
13	26.8±1.1	1.2±0.1
20	59.4±1.1	1.0±0.1

[a] μl CO_2 h^{-1} g^{-1} DM. Respiration was conducted at 42°C at all moisture levels.

[b] Ratio of vol % CO_2/vol % O_2. Values are averages and standard deviations for four determinations.

3.3. Moisture effects on respiration

The effect of seed moisture on respiration rates and RQ values are shown in Table 2. These measurements were made on seed respiring at a constant temperature (42°C), but at different moisture levels. The effect of seed moisture was more dramatic than the effect of temperature, since a three-fold increase in moisture produced a 148-fold increase in respiration rates (Table 2). Increased moisture levels also had a similar effect on maize seed respiration.[7]

4. Discussion

The procedures described above offer an easy way to measure sunflower seed respiration and respiratory quotients in which seed properties such as moisture and seed-mass temperature can be maintained at various levels with some degree of reliability. Figures 4 and 5 also demonstrate that respiration rates and RQ values can be easily measured in a 4–6 h period with high moisture sunflower seed. The time required to measure maize respiration by manometric techniques took several days.[7] Although longer times (≥24 h) were needed to measure low moisture sunflower seed (7%), accurate data were obtained because the apparatus is a closed system. Although no data are

shown, the apparatus and procedures used to measure sunflower seed respiration rates and RQ values were also successfully applied to soya beans and fescue grass seed.

Since Carbosieve S-II is an activated charcoal, it can be reconditioned after several months of use by heating to 225°C overnight with a moderate carrier gas (He) flow rate. The g.c. columns for this study have been used for over 2 years with no loss in resolution.

Seed respiring at 32 and 42°C (13% moisture) may be utilising carbohydrates as an energy source since measured RQ values were near unity (Table 1). The higher RQ values obtained from seed respiring at 51°C may suggest that O_2 availability has become limiting because of temperature effects since O_2 solubility in water (cell sap) is about 22% less at 50°C than at 25°C.[10] Previous reports also indicate that high RQ values are associated with anaerobic respiration.[8]

RQ values were essentially unity at seed moisture levels of 10, 13 and 20%, and would suggest these seed utilised carbohydrates as an energy source (Table 2). However, seed at 7% moisture yielded RQ values much higher than unity and may indicate O_2 uptake could be impeded by low seed moisture.

Sunflower seed exposed to reduced O_2 tensions of 14–15% for short periods (1–2 h) had no adverse effect on the seed since germination was 95%. Some seed samples were allowed to reduce O_2 levels to 1.5% and increase CO_2 levels to 20% and were kept in this environment for 24 h. Under these conditions, seed germination was still 95% and suggests that sunflower seed can withstand very low O_2 and high CO_2 levels without affecting seed viability. Maize seed also held at low O_2 tensions for several days did not affect germination.[7]

Mature, high-oil type sunflower seed can contain 53% (DM) of storage lipid.[11] Based on this study, carbohydrates were preferentially utilised as an energy source during seed respiration since most RQ values were unity. The fact that RQ values of 0.7 were not observed may suggest that lipids were not utilised to any extent during seed respiration. Perhaps reserve lipids of sunflower seed are only used during germination.

Acknowledgement

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